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ORIGINAL PAPER

The anti-inflammatory and wound healing properties of honey

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Abstract Honey is a natural product produced by bees and has been used for thousands of years as a medicinal agent and dietary supplement. It is known to cure a wide variety of ailments and can be used as a potent anti-inflammatory and wound healing agent. These vital bioactivities of honey are far less well known than its antibacterial, antioxidant, and any other biological activities. Many clinical trials have been reported and revealed that, when honey is applied to wound, there is a decrease in inflammation and will have a soothing effect. There is much evidence for the anti-inflammatory and wound healing effects of honey in terms of publications in modern medical and scientific journals. The exact mechanism of anti-inflammatory activity and wound healing property of honey has yet to be demonstrated. Possibly there are several mechanisms of action. There are also some reports where honey exerts negligible side effects. The article focuses on the components of honey involved in its anti-inflammatory effect, possible mechanism of action, properties of honey responsible for its wound healing activity, and its adverse effects. Overall the review presents the evidence and explanation for the anti-inflammatory and wound healing properties of honey.

Keywords Honey · Anti-inflammatory activity · Mechanism of action · Wound healing · Side effects

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Introduction

Human beings have been consuming honey since immemorial time, and it is now also being used in various food products and beverages as an additive. Honey samples vary in flavor, which is entirely based on the source of the nectar collected, and accordingly, various types and grades of honey are available. Along with the usage in foods and beverages, honey is also being used in long-standing medicinal traditions to treat various ailments and is being considered as a natural cure for a wide variety of diseases (Fig. 1). It has been used for thousands of years as a medicinal agent and dietary supplement. It has been successfully used to heal wounds [1], burns [2], and periodontitis also [3].

Honey is a sweet food produced by honey bee. They produce honey by collecting the nectar from various flowers and convert it into honey by various biochemical processes. The contents of honey vary according to nectar source, but generally consist of sugars, vitamins, minerals, and bee proteins along with some phytochemicals [4]. Honey bees transform nectar into honey by regurgitation and evaporation. They store it as a primary food source in wax honey combs inside the bee hive. Honey gets its sweetness from the monosaccharides fructose and glucose and has approximately the same sweetness as that of granulated sugar. It has attractive chemical properties for baking and distinctive flavors. Due to this characteristic, some people prefer to use honey over sugar or other sweetening agents. The water activity of honey is found to be 0.6, and because of its low water activity, most microorganisms do not grow in honey [5]. But sometimes honey may contain dormant endospores of the bacterium *Clostridium botulinum*, which can be harmful to infants, as the endospores can transform into toxin-producing bacteria in the immature [nonacid] stomach and intestinal tract of infants which then leads to illness and even sometimes death [6].

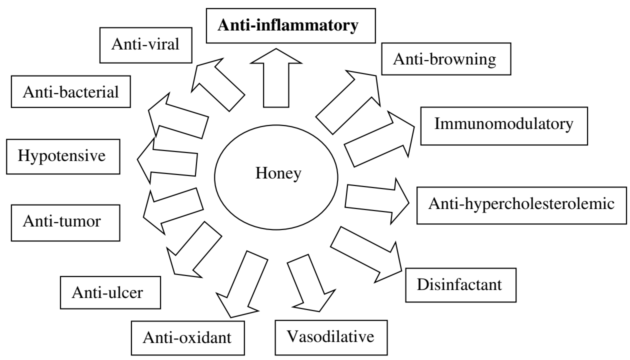


Fig. 1 Biological activities of honey

A few review articles are available in the literature, but none give the details about mechanisms of action or side effects when honey is used as an anti-inflammatory agent. The review article by Effendy focuses only on tualang honey [7]. A review by Ahmed and Othaman limited to a comparison of medicinal effects of tualang and manuka honeys [8]. Markovic et al. [9] reviewed the literature on biological activity and medicinal importance of honey. Cooper and Gray reviewed information on the mode of action of manuka honey, compared and contrasted with silver, to show that manuka honey is an effective alternative antibacterial product to silver for the prevention and management of wound infection [1]. Patel et al. [10] reviewed the pharmacological and bioanalytical aspects of galangin, a flavonol found in honey. The review by Eteraf-Oskouei and Najafi covers the composition, physicochemical properties, and the most important uses of natural honey in human diseases [11]. Recent review by Stewart et al. [12] summarizes the wound healing properties of honey. Few review articles are reported in the literature which reveals the wound healing property of honey [13, 14]. The increased number of research publications revealing the various uses of honey in biological applications, yet systematic review articles, has been critical of the design of some of those studies. None of the above are focused on the anti-inflammatory effect of honey. The present review outlines the components of honey responsible for its anti-inflammatory effect, mechanisms of action, side effects, and its wound healing properties.

Inflammation and anti-inflammation

Inflammation

Inflammation is a condition deriving from tissue response to trauma or pathogenic agents. It is a defensive way of response by an organism/tissue to remove the injuring stimuli, such as pathogens, damaged cells or irritants [15]. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli. The indications of acute inflammation are pain, heat, redness, swelling, and inability to function. If the acute inflammation is not cured early and prolonged for days, it is called chronic inflammation. The chronic inflammation leads to a progressive shift in the type of cells present at the area of inflammation and is recognized by simultaneous destruction and healing of the tissue from the inflammatory process. The chronic inflammation results in nonhealing of wounds [16] and will cause pain, ulceration, scarring, and fibrosis. Chronic inflammation can lead to other diseases such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and sometimes even cancer [e.g., gallbladder carcinoma], because the continuous destruction in the tissue would compromise the survival of the organism.

Cells involved in the inflammatory process

There are many players involved in the inflammatory process. Pro-inflammatory cytokines [such as TNF-α, IL-1, IL-6] play an important role in the process; for example, activated macrophages produce TNF-α in response to pathogens, which plays an essential role in host defense mechanism through limiting the spread of pathogenic organisms into the circulation [17]. Following are the cells involved in the inflammatory process:

(i) Monocytes: Monocytes are produced in the bone marrow. In response to acute damage or entry of foreign bodies, monocytes enlarge and synthesize enzymes in increased quantity, which are used to help break down the foreign body. (ii) Platelets: These are the cells which initiate the wound healing process, which travel to any wounded area, where through interaction with mature collagen, are activated, causing agglutination. (iii) Leukocytes: The function of leukocytes is phagocytosis, i.e., engulfment and digestion of microorganisms such as bacteria, fungi, and viruses in the blood. Leukocytes include lymphocytes, neutrophils, natural killer cells, monocytes, and macrophages. (iv) Fibroblasts: These are accountable for the synthesis of most of the collagen and elastin, thereby playing an important role in wound healing. (v) Metalloproteinases: The release of matrix metalloproteinases actively breaks down proteins and inhibits growth factors. Metalloproteinases help in removing the damaged extracellular matrix [18].

Anti-inflammation

Anti-inflammation is the positive response of a treatment that reduces inflammation, which affects the central nervous system, whereas wound healing is a process of tissue regeneration that includes inflammation as an early step.

Generally, wounds progress through three distinct phases of healing: acute, proliferative, and remodeling. Sometimes these wound healing processes are inhibited by drug therapy which delays the wound healing process. Therefore, new anti-inflammatory agents that do not inhibit these processes could be used. A natural product such as honey is the best replacement for this purpose, since it does not produce any side effects.

Honey and its anti-inflammatory property

Honey possesses quite a large number of therapeutic properties, a few of the major ones including antioxidant and antimicrobial properties, as well as anti-inflammatory activity [19]. Honey has been used mostly for topical treatment of infections as a medicine and now the wound healing properties of honey have been rediscovered [20, 21], which is being used to cure infected wounds and inflammations. The primary focus of wound treatment therapy is to kill the infectious microorganisms present in the wound and to remove any dead tissue that may provide a favorable environment for the growth of microorganisms. Inflammation not only causes problems such as making the wound uncomfortable and difficult to manage, but also prevents the tissue from repairing the wound through the healing processes. Honey rapidly cleans the wound and maintains hygienic condition in the infected area by releasing the dead tissue and destroying the bacteria, thereby reducing the inflammation, as well as stimulating growth of various types of cells and tissues involved in the generation of new tissue to repair the wound and infected tissue.

Quite a number of clinical trials have reported reduced symptoms of inflammation following application of honey to wounds [22–24], some of which also observed a soothing effect after application to wounds and burns [25]. When wounds were dressed with honey, there was a decrease of exudates, which is a good sign for managing inflamed wounds [25–28]. The anti-inflammatory action of honey also reported decreased scarring [22, 29–31]. In addition to these clinical observations, animal experiments revealed that honey application results in the reduction of inflammation compared with various controls. In histological studies, honey resulted in a reduction in the number of inflammatory cells present in burned tissue [20] and in full-thickness wounds [24, 32]. These results clearly indicate that components of honey other than sugar are involved in an anti-inflammatory effect [20]. The anti-inflammatory properties of honey are shown to be limited not only to the removal of stimuli for inflammation, or just wound cleaning, but also (as shown experimentally) to a reduction in the amount of bacteria present in the wound [32, 33], which indicates that the anti-inflammatory effect of honey is not only because of its antiseptic nature. Studies by Oryan and Zaker [32], where they observed the decrease in inflammation in wounds when honey was applied as medication, indicate that honey has a direct anti-inflammatory effect. The observed anti-inflammatory effect of honey was not due to the removal of inflammation-inducting bacteria but due to the properties of the honey itself. In a clinical trial, histological examination of biopsy samples revealed that there was a decrease in the number of inflammatory cells present in burns when dressed with honey compared with silver sulfadiazine dressing [24], providing further evidence for the anti-inflammatory activity of honey. A decrease in plasma prostaglandin concentrations in normal individuals was also observed when honey was given orally [34]. When honey was applied on localized swelling, redness, pain, and heat associated with inflammation, a reduction in inflammation was observed [35].

Irrespective of its origin, it is clear that honey exhibits anti-inflammatory effects. Yemeni sidr honey [36], gelam honey [37, 38], manuka honey [39, 40], tualang honey [7, 8, 41], chestnut honey [42], and buckwheat honey [43] all exhibit anti-inflammatory effect. Karuppannan et al. [41] evaluated the anti-inflammatory (clinical and histopathological) and antioxidant effects of tualang honey versus conventional treatment in the eyes of rabbits. They chemically induced the inflammation to the eyes of rabbits using an alkali solution. The research found no significant difference in clinical inflammatory features between honey-treated and conventionally treated subjects at different examination times, implying that honey has the same effects as that of the conventional treatment in treatment of rabbit eye injury.

Kassim et al. [44] found that gelam honey inhibits lipopolysaccharide-induced endotoxemia in rats through the induction of heme oxygenase-1 and the inhibition of cytokines, nitric oxide [NO], and high-mobility group protein B1. They found that honey reduced cytokine and NO levels while increasing heme oxygenase-1 levels. These observations support the hypothesis that honey can be used as a natural compound for the treatment of a wide range of inflammatory diseases. Recently, Liu et al. [45] compared the antioxidant, antimicrobial, and anti-inflammatory properties of honeys from different floral sources. They showed that honey inhibits the secretion of IL-8 and in turn prevents the formation of inflammation as well as possibly tumor metastasis. Similarly, Chepulis and Francis [46] carried out initial investigations on the anti-inflammatory and antioxidant activity of α-cyclodextrin-complexed manuka honey. They assessed the anti-inflammatory activity of the honey by measuring the inhibition of neutrophil TNF-α secretion. They observed both inhibition and stimulation of TNF-α, from which it can be concluded that the anti-inflammatory properties of honey may be due to the

formation of cyclodextrin-based complexes of honey, but this may differ depending on MGO (methylglyoxal) content and presence of other factors. Song et al. have studied the anti-inflammatory effect of caffeic acid phenethyl ester (CAPE), a biologically active ingredient present in propolis of honey. They evaluated the anti-inflammatory and antioxidant effects of CAPE on cultured human middle ear epithelial cells by measuring the levels of pro-inflammatory cytokines (TNF-α and COX-2) and found that CAPE significantly inhibited H_2O_2-induced up-regulation of TNF-α and COX-2 expression in a dose- and time-dependent manner. ROS accumulation induced by H_2O_2 stimulation was decreased by CAPE pretreatment. These observations suggest that inflammation induced by H_2O_2 can be inhibited by CAPE via inhibition of the expression of pro-inflammatory cytokines such as TNF-α and COX-2 [47]. In another study, the effect of honey against metanil yellow-induced liver damage is related to its antioxidant/anti-inflammatory properties which attenuate the activation of NF-κB and its controlled genes such as TNF-α and IL-1β [48]. This showed that the antioxidant/anti-inflammatory effect of honey reduced the oxidative and down-regulated the inflammatory markers.

It is well known that thrombin, hyperglycemia, and ROS play a vital role in pathogenesis of cardiovascular disease. In view of this, Ahmed et al. [49] studied the direct effect of thrombin on ROS production by human neutrophils and rodent macrophages, investigating the effect of honey on bovine thrombin-induced ROS production from phagocytes. They found that phagocytes produce ROS, and this ROS may take part in the exaggeration of the inflammatory response at the site of atheromatous plaques, upon activation with bovine thrombin. Natural honey suppressed the bovine thrombin-induced phagocytic oxidative burst indicating that honey possesses anti-inflammatory activity.

Zare et al. [50] studied the anti-inflammatory effect of subcutaneous honey bee venom injection on Wister rats. Based on the experimental results, they concluded that honey bee venom did not reduce inflammation. These studies revealed that honey and its components are responsible for the anti-inflammatory effect, but not bee venom.

Flavonoids are one of the major components of honey and have been known for years to be novel therapeutic agents for the reduction of deleterious effect of neuro-inflammation. Neuro-inflammation is a major contributor to the pathogenesis of age-related neurodegenerative disorders such as Alzheimer's or Parkinson's diseases [51]. A major indication of neurodegeneration is the presence of activated microglia, which may contribute to further neurodegeneration through the release of pro-inflammatory and/or cytotoxic factors such as IL-1β, TNF-α, NO, and ROS [52]. Candiracci et al. [53] studied the anti-inflammatory effect of honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells and found that the flavonoid extract of honey significantly inhibited the release of pro-inflammatory cytokines TNF-α and IL-1β. They also found that due to honey flavonoid extract, the expressions of inducible nitric oxide synthase (iNOS) and the production of ROS were also significantly inhibited. Hence according to these studies, flavonoid extract of honey is a potent inhibitor of microglial activation, making it a potential preventive therapeutic agent for neurodegenerative diseases involving neuro-inflammation.

Van den Berg and co-workers have studied the antioxidant and anti-inflammatory activities of buckwheat honey [43]. The tested honey samples showed the inhibition in the bioassay for ROS produced by activated human polymorphonuclear neutrophils (PMNs). Among various honey samples employed for the study, buckwheat honey showed a comparatively remarkable response as an antioxidant and anti-inflammatory agent, possibly due to large amounts of antioxidant phenolic constituents which may also have antimicrobial wound healing product. Osteoporosis associated with various stimuli such as antioxidant and anti-inflammation can be treated with tualang honey with minimum side effects as the tualang honey exhibits antioxidant and anti-inflammatory properties which can act as a free radical scavenger, reducing the oxidative stress level as well as inhibiting pro-inflammatory cytokine [7].

Bean [54] carried out research on the anti-inflammatory activity of manuka honey. She carried out an in vitro assay to measure the anti-inflammatory effect of honey and characterize this activity. There are many anti-inflammatory agents in honey and MRJP-1 (major royal jelly protein-1) is one among them. It was found that honey reduced phagocytosis in activated THP-1 cells and that certain manuka honey samples had a superior ability to other types of honey to do this. MRJP-1 and MRJP-3 were identified as being present in the fraction of manuka honey found to hold this activity.

Mechanism of anti-inflammatory action of honey

Enzymes convert sucrose into a simple and soluble mixture of monosaccharides. The sugar molecules in the honey solution bind to free water molecules (Fig. 2), which means that there is no water available for microbes to use, preventing their survival. The enzyme glucose oxidase converts glucose into gluconic acid, making the honey too acidic for microbes to grow and survive. The H_2O_2 produced as a by-product of this reaction acts as a sporicidal antiseptic that sterilizes the honey. These factors help greatly in the preservation of honey in the comb and are also useful in

diminishing microbial growth when honey is applied to an infected area or to a wound. The schematic representation of this process is outlined in Fig. 3.

The exact mechanism of anti-inflammatory action of honey has not been demonstrated so far, but it has been suggested that honey inhibits prostaglandin synthesis, which is often responsible for the observed characteristic heat, itchiness, and pain commonly associated with inflammation [55]. It has been proven by Reth [56] and Reichner et al. [57] that H_2O_2 stimulates inflammation by penetrating through the cell membrane and entering the cell nucleus. It has been observed that honey decreases the levels of ROS [58], thereby promoting wound healing. The antioxidant content in honey was thought to be responsible for this reduction in ROS production [43].

There are several possible mechanisms of action for the anti-inflammatory activity of honey. Kassim et al. [59] proposed that honey extract may cause inhibition of NO production by macrophages as the possible mechanism. It has also been reported that the anti-inflammatory effect of honey is also due to inactivation of ROS produced in the respiratory burst of phagocytes and inhibition of their production [49, 60]. As it is already well known that inhibition of ROS reduces inflammation, and the same is achieved when honey is applied to the infected part of the body. The inhibition of ROS production by honey is achieved by various cells, including zymosan-activated neutrophils [50], thrombin-activated neutrophils [52], zymosan-activated neutrophils, monocytes and macrophages [61], and zymosan-activated monocytes primed with lipopolysaccharide [49]. Literature studies have revealed that many experiments have reported that honey reduces edema and

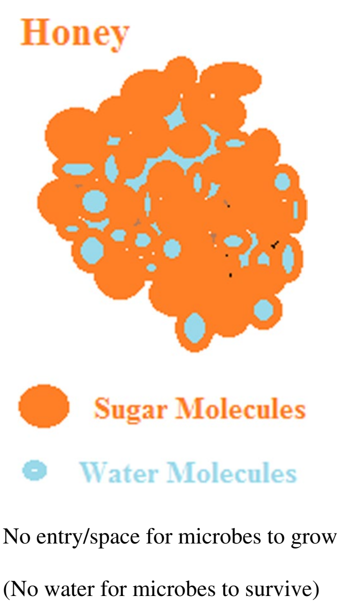
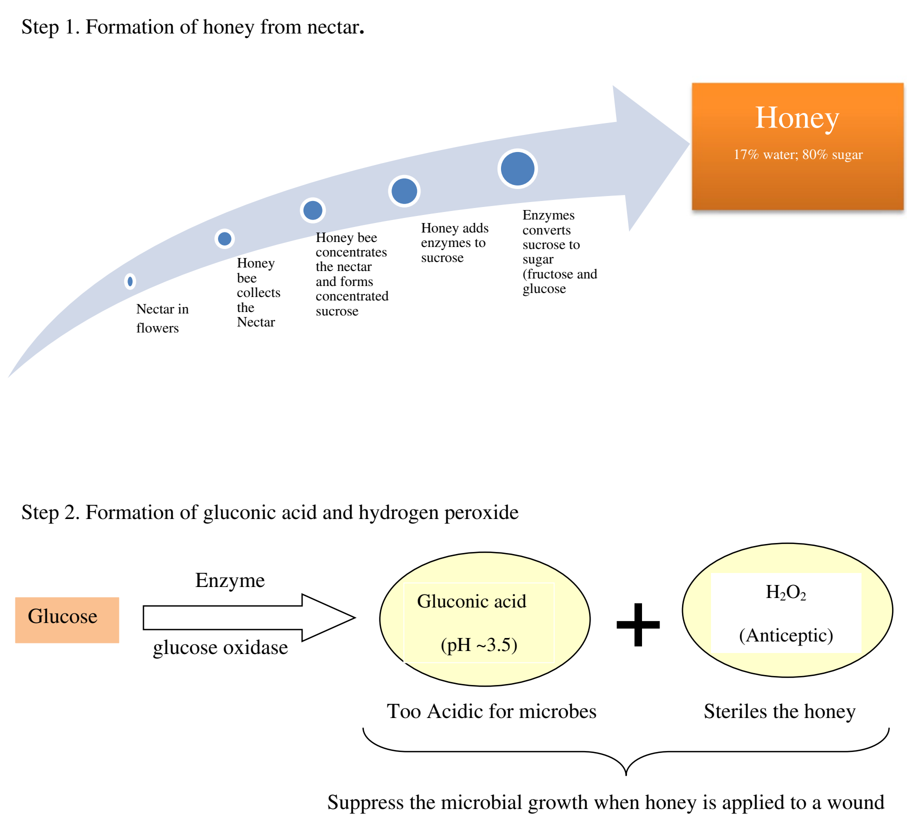


Fig. 2 Physical structure of honey

Fig. 3 Schematic representation of mechanism of anti-inflammatory action of honey



exudates levels, minimizing scarring and having a soothing effect when applied to inflamed wounds and burns [28, 42, 62].

The anti-inflammatory effects of honey can be summarized by several mechanisms of action: (a) inhibition of ROS formation [50], (b) inhibition of leukocyte infiltration [63], (c) inhibition of cyclooxygenase-2 (COX-2) and iNOS expression [64], (d) inhibition of matrix metalloproteinase-9 (MMP-9) production in keratinocytes [65]. Phenolic compounds (including flavonoids) are shown to be primarily responsible for the anti-inflammatory effects of honey [49, 59, 68]. Chrysin, a flavonoid found in honey, has been shown to have effective anti-inflammatory effect [67, 68]. It has been reported that chrysin suppresses lipopolysaccharide-induced COX-2 expression through the inhibition of nuclear factor for IL-6 DNA-binding activity [68] and inhibits the release of NO and pro-inflammatory cytokines such as TNF-α and IL-1β [69]. Majtan et al. [65] identified two flavonoids in aqueous extract of honey, namely apigenin and kaempferol, as suppressors of TNF-α-induced MMP-9 expression in HaCaT. Their findings are in line with Palmieri et al. [69] where apigenin inhibited TNF-α-induced MMP-9 expression via modulating Akt signaling in endothelial cells. It has been also found that apigenin effectively inhibited IL-1β-induced MMP-9 mRNA expression in osteoblasts [70]. So far a little work has been carried out regarding the effects of kaempferol on MMP-9 induction and expression; however, a theoretical study revealed that kaempferol is a potent inhibitor of MMP-2 and MMP-9 activities [71]. Also, Majtan et al. [65] revealed that honey-mediated attenuation of TNF-α-induced MMP-9 expression in HaCaT, suggesting that the flavonoid content of honey is able to down-regulate the expression of MMP-9, a key inflammatory mediator responsible for destructive effects in chronic wounds.

Wound healing property of honey

The unique antibacterial, anti-inflammatory, and antioxidant properties of honey were found to contribute to wound healing, especially in ulcers and burns [72]. A large number of publications and text books in the twenty-first century evidenced the properties of honey in wound management [in veterinary and human] and other medical applications of honey [73]. Carnwath et al. [74] have assessed the antimicrobial activity of a number of honey types against common equine wound bacterial pathogens. The results demonstrated that certain varieties and sources of honey are effective at inhibiting microbial growth on wounds in vitro at very low concentrations. It has been observed that the effectiveness of natural commercial honey in combination with a hydroalginate and off-loading in managing diabetic foot ulcers is possible at primary care level [75]. Medicated monuka honey was found to be effective in treating exomphalos major [76].

Properties of honey that contributes to its wound healing effect

Deodorizing property of honey

There are reports which revealed the deodorizing property of honey [28, 77]. Dressing the malignant wound with honey was found to remove the malodor, which is not controlled by any other treatment. Ammonia, amines, and sulfur are the malodorous chemicals produced in wounds by bacteria. These chemicals are formed by the metabolism of amino acids and tissue proteins. Instead of amino acids, bacteria metabolizes glucose present in the honey, and hence, in the presence of honey, the malodorous substances will not be generated [78].

Viscosity of honey

When honey is applied to a wound, its high viscosity provides a physical barrier to infection of wounds from foreign contamination, the effectiveness of which is increased by the antibacterial activity of the honey. This unique characteristic is particularly useful in highly exudative wounds such as burn wounds, where avoiding occluding is required. Skin grafts, infected with the bacteria *Pseudomonas spp,* can be treated with honey dressings. This suggests that the application of honey on surgical wounds can help prevent the patients from contracting nosocomial infection with methicillin-resistant Staphylococcus aureus (MRSA) [30, 70]. These features indicate that honey is likely to be used as an effective prophylactic agent.

Debriding action of honey

The medicated honey acts as an autolytic debriding agent in majority of wounds [80]. Additional advantages such as reduction in wound exudate, malodor, and pain, as well as the stimulation of new tissue growth were also observed. Wounds that are dressed with honey are rapidly debrided to give a clean granulating wound bed [27, 28, 31], also allowing necrotic tissue to painlessly lift off [23]. The debriding property of honey fastens the curing of inflammation, which is a great advantage on sloughy wounds. The usage of honey in the place of maggots ensures that the wound is not too wet or too dry, which is essential for effective healing. The debriding action of maggots is due to the activity of secreted proteolytic enzymes, but with honey there would be no direct proteolytic activity involved. The

possibility of protein-digesting enzyme activity in honey has not been investigated. Hence, honey stimulates dormant proteolytic enzyme activity in a controlled manner within the infected tissue, so as not to cause unwanted digestion of the tissue or muscle. There is a strong relationship between high protease activity and impaired wound healing, so dressings are being formed such that they inhibit and inactivate the excessive protease activity in wounds that may digest the infection [81, 82]. The debriding activity of honey may be due to the conversion of inactive plasminogen in wound matrix to the plasmin, which is the most active form of plasminogen. Fibrin clots which attach slough and eschar to the wound bed are broken down by the enzyme plasmin. This property of conversion of inactive plasminogen into active plasmin by honey could also contribute to its anti-inflammatory activity [32]. The osmotic action of honey also contributes to its debriding property by drawing out lymph from the wound tissues, thereby providing a constant supply of plasminogen to the infected tissue. The osmotic action of honey also cleans the surface of the wound bed from beneath. This would account for the removal of dirt with the dressing, which maintains hygienic conditions in dressing.

The adherence of skin grafts to wound beds can be increased by applying honey, thereby increase healing rate of wounds. Medical honey has been found to be a very effective agent in skin graft fixation, preventing negative factors of skin graft loss such as infection and graft mobility. Major advantages of this procedure are that it is time saving, easy in application, and cheap, but it should be used sterile [83].

Components of honey and their roles in anti-inflammatory activity

Honey contains at least 200 components and the majority of them are carbohydrates and water. It also contains minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals [84]. Honey also contains various types of sugars, acids, proteins, and minerals. However, some other components of honey which contribute to its anti-inflammatory activity are as follows:

(i) Hydrogen peroxide (H_2O_2): One of the main components of honey, hydrogen peroxide, is responsible for honey's anti-inflammatory properties and is a well-known antibacterial molecule [85, 86].

(ii) Glucose oxidase: An enzyme secreted into the nectar by bees when honey is made. H_2O_2 is produced by glucose oxidase enzyme by the oxidation of glucose and oxygen [87].

(iii) Gluconic acid: The pH of honey is between 3.2 and 4.5. This low pH is due to the presence of gluconic acid. During the process of ripening of nectar into honey, gluconolactone/gluconic acid is produced by the action of glucose oxidase. The pH value of around 3.2–4.5 is too acidic for the survival of many wound-infecting bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella sp*, which suggests that when honey is applied to the infected area, the chance of infection will be decreased.

(iv) Nonperoxide antibacterial components: Some honey contains components responsible for nonperoxide antibacterial activity (e.g., manuka honey). Such types of honey contain large amount of MGO [87, 88], which is responsible for nonperoxide antibacterial activity. However, the exact MGO content in honey depends on the source of the nectar which is collected by the bees [87].

(v) Major royal jelly protein (MRJP): Royal jelly is a honey bee secretion which is used for the nourishment of larvae and adult queens. Worker bees secrete royal jelly from their glands in the hypopharynx and feed it to all larvae of the colony. The composition of MRJP is fairly complex, including various proteins, amino acids, organic acids, steroids, esters, phenols, sugars, minerals, trace elements, and other constituents. In addition, seasonal and regional conditions also affect the composition of royal jelly [89, 90]. 27–41 % of proteins represent the most important components of the royal jelly and more than 80 % of royal jelly proteins are soluble proteins which are commonly known as MRJPs [91]. So far, eight major RJ proteins (MRJP1, MRJP2, MRJP3, MRJP4, MRJP5, MRJP6, MRJP7, and MRJP8) have been identified and characterized [92]. Many studies have revealed that the RJ proteins exhibit many pharmacological activities, including anti-inflammatory properties [93–96]. Keizo et al. [93] examined the anti-inflammatory effect of RJ at the cytokine level. They found that when treated with a suspension of RJ supernatant, the production of pro-inflammatory cytokines, viz TNF-α, IL-1, and IL-6 was efficiently inhibited in a dose-dependent manner. This suggests that RJs contain the factors responsible for suppression of pro-inflammatory cytokine secretion.

Relationship between antioxidant, antibacterial, and anti-inflammatory activities of honey

The main factor responsible for the anti-inflammatory activity of honey may be its antioxidant activity. It has previously been established that honey contains antioxidant

agents at significant levels [4, 97, 98] including catalysis of Fenton reaction II, which forms free radicals from H_2O_2 [99]. These free radicals help to restore more leukocytes into the site of inflammation as a self-amplification of the inflammatory response. The self-amplification is by oxidative activation of the nuclear transcription factor NF-κB, which then helps in the formation of pro-inflammatory cytokines by leukocytes [100], and excites the activity of the fibroblasts, thus including hyper granulation and fibrosis [101]. The free radicals formed from H_2O_2 are responsible for the activation of the transcription factor NF-κB [102], and this activation can be stopped by antioxidants [103]. Oxidative stress and inflammation play a vital role in the etiology of many diseases [104–106]. Reports revealed that both of them are intimately interrelated with one another since one can cause the other [104, 105]. It has been reported that intra-rectal honey administration significantly reduced myeloperoxidase activity in rats with inflammatory bowel disease [107]. This was associated with lower levels of colonic malondialdehyde with no change in nitrogen oxide (NO) content [108]. Kassim et al. [55] studied the effects of honey and its extracts in rat models of inflammation, finding that honey and its extracts inhibited NO and prostaglandin E_2 production. They also found that honey and its extracts significantly reduced the edema and pain in inflammatory tissues. This inhibition of edema and pain was found to correlate with the inhibition of NO and prostaglandin E_2 [55]. Recently, another study by Owoyele et al. [108] revealed the effects of different doses of honey on acute and chronic inflammations in rats using carrageenan, cotton pellet, and formaldehyde methods and NO production by administering nitro-L-arginine methyl ester and L-arginine. They found that administration of honey reduced paw size, granuloma weight, and arthritis in the carrageenan [108]. These clinical reports clearly indicate that honey can exert an anti-inflammatory effect through the inhibition of NO and prostaglandin E_2 production and release [108]. These antioxidant effects may contribute to its anti-inflammatory effect [109].

It is well known that honey is an antibacterial agent with broad spectrum of activity against both gram-positive and gram-negative bacteria [110]. In honey, many bioactive compounds have been identified which are associated with its antibacterial action. The commonly accepted ones include osmolarity [111], H_2O_2 [112–114], catalysis to H_2O_2 ratio [114], polyphenols [115], antioxidants and Maillard reaction products [116], antibiotic peptides [117], and MGO [87, 88]. The structural and functional diversity of these bioactive components will damage bacterial cells through various mechanisms. The osmotic effects of honey sugars and biocidal action of H_2O_2 produced in honey are the main players responsible for the antibacterial activity of honey, which are also contributing to its anti-inflammatory effect. The degradation of DNA by honey is due to the coupling chemistry between polyphenols and H_2O_2 that results in phenolic auto-oxidation and a generation of radical species [118].

Adverse effects of honey

Natural products are known to cure some ailments without any side effects, for example honey, which is very safe to use. There has been no report of any adverse effects using honey both on wounds and in ophthalmology. Most of the commonly used antiseptics can be harmful [119] including silver, which is released from nanocrystalline silver dressings [120], whereas with honey there have been no reports of any cytotoxic effects. However, reports have mentioned that when honey is applied to wounds, it causes a stinging pain [28, 31]. This is possibly due to the acidity of honey, evidenced by the application of neutralized honey causing no such effects [22]. Honey stimulates sensory neurons [121], and it is of interest that patients have been reported to experience a sensation from application of honey to their ulcers [122]. This may not be because of the direct effects of the acidity of honey, as neutralized honey could affect the ionization of some of its components and make them unable to fit in the nociceptors. It may, however, be due to the sensitiveness of these nerve endings, which are more responsive to the acidity and/or the organic components present in the honey. There are many studies which report the pain-relieving properties of honey [26, 29–31, 123–125]. In a trial by Al-Waili and Haq, they observed that the pain experienced with saline-soaked gauze and paraffin gauze was one-third more than with honey-soaked gauze dressing, but slightly more than with a hydrocolloid dressing [26]. In another trial by Dunford and Hanano, where the comfort of honey dressings on chronic venous leg ulcers was investigated, six patients experienced a transient stinging pain and eight felt a lasting pain [126]. In this trial, it was noted from the opinions of the patients that the pain was significantly decreased by the honey dressings, and the feedback from the patients with the honey dressings was quite good. In another clinical trial of honey dressings, one of the sixty patients treated with honey withdrew because the dressings caused pain [28]. Similarly, in another trial where the effect of honey dressings on chronic venous leg ulcers was studied, six patients (out of a total of forty participants) withdrew from the trial because of the pain by application of honey gauze, which was higher than average pain before the start of the honey dressing application [126]. Other major adverse effect of honey is causing infant botulism to the infants less than 1 year of age. It is known that honey is the dietary reservoir of dormant endospores of

the bacterium, *Clostridium botulinum*, which are linked to infant botulism. Hence, it is advised that honey should not be fed to infants less than 12 months of age [127] as the bacteria in the infant's gut are not fully developed. The consumption of honey during this period has been noted as a risk factor for infant botulism. Avoiding honey to infants is the best way to prevent the infant botulism. In children and adults, the normal intestinal bacteria inhibit the development of *Clostridium botulinum* and hence will not be any botulism [128].

Conclusions and prospects

The human society has been using honey for more than 4,000 years without any significant side effects, which reveals its usefulness as a potent anti-inflammatory and wound healing agent. Honey has been used from the ancient days not only as food, but also for curing various biological disorders. In an overview, it is seen that the anti-inflammatory activity of honey minimizes scarring. When honey is applied on burns, it prevents inflammation progression which could lead to further damage. The anti-inflammatory effects and wound healing properties of honey have been evidenced by experiments on animals and various clinical trials conducted. There is a good amount of published evidence for anti-inflammatory and wound healing properties of honey, and some reports propose the speculative mechanisms of action. Honey is natural product that received a growing attention from the scientists and clinicians toward its anti-inflammatory and wound healing properties. Though the exact mechanism of action is still unknown, research has focused on the antibacterial property of honey as a cause. Because of its high sugar content, honey is hygroscopic in nature which means it has a dehydrating effect that inhibits the bacterial growth, which contributes to its anti-inflammatory and wound healing properties.

Even though various researchers around the globe explored the anti-inflammatory and wound healing properties of the honey from different origins, the main component of honey responsible for these vital properties is unknown. Future research needs to focus on this point, and on the proposal of the cellular/molecular level mechanism of action of these vital bioactivities of honey. Therefore, it is necessary to explore further for the anti-inflammatory and wound healing effects of honey. More research is required to reveal the effects of kaempferol on MMP-9 induction and expression. So far, the protein-digesting activity of enzyme in honey has not been investigated. The research on the anti-inflammatory activity of honey needs to solve this problem also. Studies are also needed to focus on whether honeydew honey, which contains certain flavonoids, would also be able to contribute to the anti-inflammatory effects of honey.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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